Chronic Lymphocytic Leukemia: A Niche for Flavopiridol?

□□*Commentary on Byrd et al., p. 4176*

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Cyclin-dependent kinases (cdks) are key regulators of orderly progression through the cell cycle and of RNA transcription. Inhibition of their activity *in vitro* can cause cell cycle arrest at the G_1-S or G_2-M boundaries (1, 2). Flavopiridol, a synthetic flavone related to a natural product of the bark of a plant native to India, has generated intense interest as the first cdk inhibitor to enter clinical trials. Flavopiridol selectively inhibits multiple cdks by blocking ATP binding and by blocking their phosphorylation by cdk7 (1, 2). Flavopiridol also decreases cyclin D1 levels (3) and inhibits gene transcription by blocking cdk9, which phosphorylates RNA polymerase II (4).

Initial observations in cell culture found that flavopiridol at 150 to 200 nmol/L is cytostatic, causing reversible cell cycle arrest (5). Flavopiridol is also cytotoxic to both cycling and noncycling A549 lung cancer cells, however (6, 7). Hematopoietic cells, including B cell lines and chronic lymphocytic leukemia (CLL) cells *in vitro*, are particularly susceptible to early apoptosis induced by flavopiridol, with LC_{50} 1.15 μmol/L at 4 hours and 100 to 400 nmol/L at 24 hours of exposure (8, 9). Solid tumor cell lines often require prolonged exposure to higher flavopiridol concentrations (0.5-1 μmol/L for 24-72 hours) for equivalent cytotoxicity (6, 7).

The observation that the cytotoxicity of flavopiridol is enhanced by prolonged exposure *in vitro* led to two phase I clinical trials of a 72-hour continuous infusion every 2 weeks (10, 11). The maximum tolerated dose was 40 to 50 mg/m^2/day continuous infusion for 3 days, with a dose-limiting toxicity of secretory diarrhea. Pharmacokinetic data showed mean steady-state plasma concentrations of >270 nmol/L, similar to the *in vitro* concentration required for cdk inhibition (10–12). Objective responses were noted in renal cell and gastric cancer, and minor responses in non–Hodgkin's lymphoma, renal cell, and colon cancer.

These encouraging phase I results led to phase II studies of this dose and schedule in gastric, lung, colon, and renal cell cancers (13–16). No objective responses were observed in gastric, lung, or colon cancers, and only two responses (6%) in renal cell cancer, despite steady-state flavopiridol concentrations >200 nmol/L in the gastric, lung, and renal cell studies (13, 15, 17). The same schedule in mantle cell non–Hodgkin's lymphoma also produced no objective responses and led to early termination of the study (18). Efforts to identify biological activity of flavopiridol found no change in the number of peripheral blood mononuclear cells (13) and no inhibition of stimulated proliferation of peripheral blood mononuclear cells *in vitro* (14).

The lack of biological activity of flavopiridol in a 72-hour continuous infusion was clearly discordant with its *in vitro* potency in inducing cdk inhibition and cell cycle arrest at concentrations achieved *in vivo*. Inducing cytotoxicity in solid tumor cell lines *in vitro* has often required significantly higher flavopiridol concentrations, however (2, 5). Furthermore, in HL60 xenografts, 72-hour continuous infusion flavopiridol has little or no activity, whereas daily i.v. bolus flavopiridol potently inhibits the xenografts, leading to prolonged complete remissions (19). The continuous infusion schedule produced mean steady-state concentrations of 427 nmol/L, whereas the bolus schedule achieved peak concentrations of 7 μmol/L at 2 minutes, declining to 0.1 μmol/L by 8 hours (19). The bolus schedule had potent effects on hematopoietic tissues, inducing dose-dependent leukopenia, atrophy of lymphoid organs, and inhibition of mitogen stimulation of peripheral blood mononuclear cells (19).

A phase I human study of 1 hour i.v. bolus flavopiridol identified the maximum tolerated dose as 50 mg/m^2/day for 3 days, with dose-limiting toxicity now grade 4 neutropenia (20). The median peak plasma concentration on this schedule was 3.2 μmol/L, approaching that in the xenograft model (21). Although no objective responses were observed, 24% of patients had stable disease (20). A phase II study of flavopiridol with this dose and schedule in mantle cell non–Hodgkin's lymphoma resulted in 11% partial responses and 71% stable disease, with some evidence of additional transient lymph node regressions (22). These results are significantly improved over the 72-hour continuous infusion and suggest some level of activity.

The rationale for studying flavopiridol in CLL is high. In CLL, lymphocytes accumulate at least in part due to a defect in apoptosis. Flavopiridol induces apoptosis in CLL cells *in vitro* in a p53-independent, caspase-dependent manner (9), and has been reported to decrease expression of antiapoptotic proteins including Mcl-1 and X-linked inhibitor of apoptosis (23, 24). If flavopiridol can induce cell death *in vivo* in the absence of p53, it could become an effective therapy for CLL patients with poor prognostic features, particularly mutations of 17p and the p53 pathway.

In this issue of *Clinical Cancer Research*, Byrd et al. report the results of two sequential phase II studies of flavopiridol in previously treated CLL, done by the Cancer and Leukemia Group B. The first study, using 50 mg/m^2/day continuous infusion over 72 hours, resulted in no objective responses and only 27% stable disease. The second study, using the 50 mg/m^2/day bolus over 1 hour for 3 days, resulted in 11% partial responses and 53% stable disease. All four responding patients in this study had fludarabine-refractory disease. One patient

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Received 4/15/05; accepted 4/15/05.

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who had a partial response developed acute tumor lysis syndrome, which was successfully managed medically.

Thus, similar to what was seen in mantle cell non–Hodgkin's lymphoma, Byrd et al. find that flavopiridol has minor activity in CLL when given by i.v. bolus but not by continuous infusion. The observation of tumor lysis syndrome is particularly notable and suggests the potential for greater efficacy than seen in this study. It is unfortunate that no pharmacokinetic data are available to correlate with response and tumor lysis. However, prior experience with flavopiridol shows that the i.v. bolus schedule results in significantly higher peak concentrations (20, 21). Why these higher concentrations are apparently needed for biological activity is unclear, given that low concentrations inhibit cdk activity and lead to cell cycle arrest *in vitro*. Possible explanations include inhibition of activity in human plasma, possibly due to plasma protein binding, unusual drug metabolism or an endogenous inhibitor, or the need for higher concentrations to reach an unknown target required for cytotoxicity *in vivo*. Preliminary data from the authors of these trials and others does in fact suggest that 92% to 95% of flavopiridol is protein-bound in human plasma, but not in fetal calf serum used *in vitro*, resulting in an increase in the LC_{50} for CLL cells *in vitro* from 0.67 to 3.5 μmol/L at 1 hour, and from 0.12 to 0.47 μmol/L at 24 hours (12, 25). These concentrations have not been reliably achieved in clinical studies, suggesting that increased protein binding may at least contribute to the dose and schedule dependence of flavopiridol activity *in vivo*.

Byrd et al. have gone on to test this hypothesis in relapsed CLL patients in a subsequent phase I study using flavopiridol given in a 30-minute bolus dose followed by a 4-hour infusion, designed based on pharmacokinetic modeling to achieve and sustain micromolar concentrations for several hours (25). As reported at the American Society of Hematology meeting last December (26), a remarkable 41% response rate was observed in 22 evaluable patients; eight of nine responders had fludarabine-refractory disease, bulky lymph nodes, and either 11q or 17p deletion. Concomitant with this activity, however, was significant tumor lysis syndrome, which proved to be the dose-limiting toxicity, resulting in one patient death, multiple patients requiring dialysis, and all patients requiring aggressive inpatient medical management. This dramatic improvement in tumor response with a pharmacokinetically designed schedule suggests that tumor response and tumor lysis are directly tied to achieved drug concentrations, but confirmation of this hypothesis will await the full publication of this study and its accompanying pharmacokinetic data.

These results, emerging from persistent and careful characterization by Byrd and colleagues, suggest significant activity of flavopiridol in refractory CLL. Many questions remain unanswered, however, and finding the answers will greatly enhance our insight into mechanisms of drug response and cell death in CLL and possibly other cancers. For example, will individual pharmacokinetic variables correlate with likelihood of tumor lysis and tumor response? Do genetic polymorphisms in flavopiridol glucuronidation, previously described to impact the incidence of diarrhea (17), also affect drug concentration, tumor lysis, and tumor response? Is plasma protein binding the sole reason why higher than expected flavopiridol concentrations are required for activity? What is the critical target required for flavopiridol-induced apoptosis in CLL cells *in vitro*, and is it targeted in responding patients? Does this target explain the apparent rapidity of CLL cell death and tumor lysis seen in the most recent trial? Is flavopiridol effective in high-risk CLL patients with 17p deletion or p53 mutation, and does its putative target explain this effectiveness? The answers to these questions will not only provide significant insight into the fundamental biology of CLL, but are also particularly critical for further clinical development of flavopiridol, which will require optimizing its safety as well as its efficacy.

Finding a niche for flavopiridol in CLL therapy may depend on developing predictors of which patients are most likely to develop tumor lysis syndrome, or optimizing the dosing schedule to maintain activity with increased safety. If these prove difficult but significant disease response is confirmed in subsequent studies, particularly in high-risk patient populations, flavopiridol could prove useful for clearance of residual disease following chemotherapy. Alternatively, lower doses of flavopiridol may be able to sensitize CLL cells to the effects of chemotherapy without inducing life-threatening tumor lysis (27, 28). Combination regimens studied thus far have found significant but likely manageable toxicity, with more activity than in studies of single-agent flavopiridol (2, 29, 30).

Pharmacokinetically designed schedules of flavopiridol administration are currently being tested in the treatment of other malignancies (2). In mantle cell lymphoma, the older i.v. bolus schedule showed activity similar to that seen in CLL (22). Given that mantle cell lymphoma overexpresses cyclin D1, and flavopiridol decreases levels of cyclin D1, the rationale for studying an optimized schedule in mantle cell lymphoma is high. In solid tumors, although few responses have been seen in phase II trials, prolonged stable disease has been observed and correlates with higher plasma concentrations of flavopiridol (15). Furthermore, higher flavopiridol concentrations not yet reached in clinical trials are likely required *in vitro* for cytotoxicity in solid tumors, possibly due to a different target than in CLL. Chemotherapy may be able to sensitize solid tumors to the effects of flavopiridol *in vitro* (27, 28), and tumor responses have been seen in phase I studies of flavopiridol in combination with gemcitabine and paclitaxel (2, 30). Ongoing and future research will be required to determine whether flavopiridol has a role in the therapy of solid tumors. Although much work remains to be done in CLL as well as in other malignancies, the development of flavopiridol to date suggests that systematic pharmacokinetic and mechanistic analysis in human studies is essential to identify the true potential of new drug candidates, even novel therapies for which a putative target is thought to be known *in vitro*.

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